Bacterial-Induced Systemic Resistance in Soybean (*Glycine Max* L. Merrill) Upon Challenge Inoculation with Fungal Pathogens

A

SYNOPSIS

SUBMITTED FOR THE PARTIAL FULFILMENT OF

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Introduction-

In the present scenario of agro-world the first priority of the cultivator is to produce a healthy plant, i.e. plant without any infectious disease and to gain high yield in any adverse condition. Control of plant disease is a pressing need for agriculture in the 21st century. Agricultural products are the primary dietary source of proteins and vitamins. Soybean (*Glycine max* L. Merrill) is one of the members of family fabaceae under order fabales. It is produced world-wide and the main producers of soybean are the United States (35%), Brazil (27%), Argentina (19%), China (6%) and India (4%) (World Soybean Production 2010).

In India soybean production is mainly confined to Madhya Pradesh, about 53 %, also known as soybean bowl of India, Maharashtra (34%), Rajasthan (8%) and other states (5%)(USDA report, 2013). Soybean possesses a very high nutritional value. It contains about 20 percent oil and 40 percent high quality protein. Soybean protein is rich in valuable amino acid lycine (5%). In addition, it contains a good amount of minerals, salts and vitamins (thiamine and riboflavin) and its sprouting grains contain a considerable amount of Vitamin C, Vitamin A is present in the form of precursor carotene, which is converted into vitamin A in the intestine (http://www.pnbkrishi.com/soybean.htm).

Soybean has been reported as world's most important source of high protein content among leguminous crops (El-Abady et al. 2008). Several pathogens like fungi, bacteria and viruses attack on the soybean plant and cause different disease leading to the great losses in the yield. Among the different diseases fungal diseases such as Alternaria leaf spot, Charcoal rot, Damping-off, *Fusarium* root rot, *Pythium* rot, *Sclerotinia* stem rot and many others affects soybean production on a large scale (Common Names of Diseases, The American Phytopathological Society). *Fusarium* rot known as *Fusarium* wilt and necrosis of root and lower of soybean caused by *Fusarium oxysporum* is one of the most destructive diseases of the crop and is very common (Hashem et al. 2009). Besides of the endogenous defense system of plant such a waxy cuticles and antimicrobial components other defense mechanisms are inducible (Bonas and Lahaye 2002), thus presenting a prospective method of reducing disease severity caused by a wide variety of pathogens (Nürnberger and Brunner 2002). According to Agrios (1988) Resistance is the ability of an organism to exclude or overcome, completely or in some degree, the effect of a pathogen or other damaging factor (Van Loon 1997) while Induced resistance is a state of enhanced defensive capacity developed by a plant when appropriately stimulated by specific environmental stimuli. There are mainly two forms of induced resistance

systemic acquired resistance (SAR) and induced systemic resistance (ISR) wherein plant defenses are preconditioned by prior infection or treatment that results in resistance against subsequent challenge by a pathogen or parasite (Choudhary 2012). In contrast to overcome the yield loss of crops due to biotic stress biological control is considered as an alternative or a supplemental way of reducing diseases in agro-ecosystem (Sharma and Johri 2003).

In the Rhizosphere which works as a protective shield for plants, root must compete for the primary need such as space, water and mineral nutrients, with invading root system of neighboring plant species and for different source of organic nutrients with soil-borne microorganisms, including fungi, bacteria and insects (Choudhary and Johri 2009). These bacteria have beneficial effects on plant growth promotion, development and protection against plant pathogens by eliciting ISR, and therefore it is termed as plant growth promoting bacteria (PGPB) (Kloepper et al. 1980). The rich diversity of the microbial world provides an apparently endless resource for this purpose. Increasing the abundance of a particular strain in the vicinity of a plant can suppress disease without producing lasting effects on the rest of the microbial community or other organisms in the ecosystem (Gilbert et al. 1993; Osburn et al. 1995) The widely recognized mechanism of ISR mediated by PGPR is competition for an ecological niche or substrate, production of inhibitory allelochemicals, and induced resistance in host plants to a broad spectrum of pathogens (Choudhary et al. 2007). Induced resistance is associated with the elevated level of the defense gene products such as peroxidase (POD), polyphenol oxidase (PPO), phenylalanine ammonia-lyase (PAL), lypoxigenase (LOX), chalcone synthase (CHS), catalase (CAT) etc. These defense genes are responsible for the prior strengthening of defense system of plant against pathogen by formation of lignin or activating salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) response. Besides of the plant growth-promoting activities and direct antifungal activity, activation of defense gene products by prior application with PGPR against a challenging pathogen is a novel strategy in plant protection.

Objectives of the study-

The main focus of the study is the elicitation of ISR in the soybean plant against fungal pathogens by the bacterial treatment.

The objectives will comprise of:

1. Isolation and functional characterization of plant growth promoting activities of bacteria
2. Molecular characterization of plant growth promoting activities of isolated bacteria
3. Characterization of soybean pathogen suppression mechanism by plant growth-promoting bacteria
4. Elicitation of induced systemic resistance in soybean through plant growth-promoting bacteria.
5. Molecular gene expression analysis of defense-related genes in soybean plant

Review of literature-

Plant growth-promoting rhizobacteria (PGPR) are free-living, soil-borne bacteria, isolated from the rhizosphere, which, when applied to seeds or crops, enhance the growth of the plant or reduce the damage from soil-borne plant pathogens (Kloepper *et al.* 1980). Rhizosphere bacteria such as *Acinetobacter, Agrobacterium, Arthrobacter, Azospirillum, Bacillus, Bradyrhizobium, Frankia, Pseudomonas, Rhizobium, Serratia, Thiobacillus* promote plant growth and yield either directly by the synthesis of substances or facilitation of the uptake of nutrients from the environment through nitrogen fixation, solubilization of phosphorus, siderophores production, phytohormones production (Kloepper *et al.* 1989; Glick *et al.* 1999) or indirectly by preventing the deleterious effects of plant pathogens on plants by production of inhibitory substances such as antibiotic, siderophore, antifungal metabolites, fungal cell wall lysing enzymes or by increasing the natural resistance of the host through ISR (Nehl et al. 1996; Cartieaux et al. 2003). Plants possess a powerful immune system as a protective guard against microbial pathogens and parasites, which is coordinated by a complex signaling network. According to the types of molecules recognized by plants as indicator of a pathogen attack, they have two types of immune system, termed PAMP-triggered immunity (PTI) and Effector-triggered immunity (ETI) (Vleesschauwer and HÖfte 2009; Eulgem and Somssich 2007).Inspite of having such a strong immune system sometimes plants get affected by some infectious microbes. Pieterse et al. (1998) have find out the signaling pathways of SAR and ISR and also found interconnection between both signaling pathways which regulate the induced defense in plant against pathogens.

SAR can be activated by exposing the plant to virulent, avirulent, and nonpathogenic microbes. In SAR accumulation of pathogenesis-related proteins (chitinase and glucanase), and salicylic acid takes place. ISR is triggered by PGPR in which accumulation of pathogenesis-related proteins or salicylic acid does not take place and it relies on pathways regulated by jasmonate and ethylene (Pieterse et al. 2001; Yan Z et al. 2002) (Fig.1). The primary components of the network are plant signal molecules—salicylic acid (SA), jasmonic acid (JA), and ethylene (ET). Research study on plant microbe interaction showing that PGPR elicited ISR in plant against different pathogens to reduce susceptibility against concern disease. It was first observed on carnation (*Dianthus caryophillus*) with reduced susceptibility to wilt caused by pathogenic fungus *Fusarium* sp. (Compant et al. 2005) and on cucumber (*Cucumis sativus*) with reduced susceptibility to foliar disease caused by *Colletotrichum orbiculare* (Wei et al. 1991).

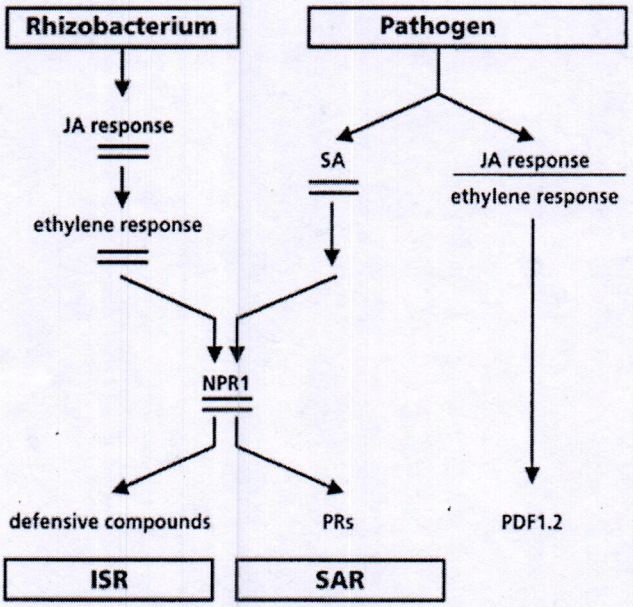

Fig.1- The pathogen-induced SAR and the rhizobacteria-mediated ISR signal transduction pathways. (Source: Pieterse et al., 1998)

In *Arabidopsis*, all three signaling molecules have been shown to activate specific sets of pathogen-inducible defense-related genes. SA is the primary molecule for SAR which activate further signaling cascade to activate gene responsible for resistance against pathogen, called pathogenesis-related (PR) gene which encode different Pathogenesis-related proteins (PRs) of families PR-2, PR-5 and PR-1, such as chitinases, β-1,3-glucanases, lypoxygenases, thoumine like proteins, anti-microbial peptides etc. (Saskia et al. 1999). NPR-1 protein encoded by *npr-1* gene play an important role in SAR establishment as it activate PRs genes after getting signal form SA accumulation (Pieterse et al. 1998). According to Vleesschauwer and HÖfte (2009), by some recent research it has proven that the volatile molecule methyl salicylate (MeSA) can act as a long-distance mobile signal for SAR. MeSA itself appears to be biologically inactive, but in the systemic tissue MeSA is hydrolysed to SA by the MeSA-esterase activity of SA-binding protein-2 (Park et al. 2007). On the other hand ISR have a more diverse and complex route to establish a higher degree of prior resistance without any infection. Besides SA, the plant growth regulators jasmonic acid (JA) and ethylene (ET) have been implicated in plant defense responses (Pieterse et al. 1998; Wasternack and Parthier 1997). Jasmonic acid and its different derivative induce the expression of genes encoding defense-related proteins, such as thionins (Pieterse et al. 1998; Epple et al. 1995) and proteinase inhibitors (Pieterse et al. 1998; Farmer et al. 1992), whereas

ethylene is involved in the expression of the pathogen-inducible genes(Saskia et al. 1999). Like MeSA, Methyl jasmonate (MeJA) also work as a volatile signal for the distal part of the plant. Expression of different defense-related gene depends on the fact that NPR -1 is getting signal from JA or ET or from both in concert. Ethylene is involved in the expression of the pathogen-inducible genes *Hel* (encoding a hevein-like protein) (Potter *et al.*, 1993), *ChiB* (encoding a basic chitinase) (Samac *et al.*, 1990), and *Pdf1.2* (encoding a plant defensin) (Penninckx et al. 1996). JA was also found to be responsible for the activation of the *Hel*, *ChiB*, and *Pdf1.2* genes as well (Thomma et al. 1998), *Pal1* gene, (Mauch-Mani and Slusarenko 1996), *Pin* gene (encoding proteinase inhibitor proteins) (Farmer and Ryan, 1992) and *Atvsp* gene (encoding vegetative storage protein) in *Arabidopsis* (Berger et al. 1995). Induced expression of the defense enzymes such as PAL, POD, PPO, β-1, 3 glucanase, LOX (Ramamoorthy et al. 2002; Choudhary 2011), CHS (Mori et al. 2001) Chitinase, ascorbate peroxidase (Seo et al.2012) reported in the PGPB treated plants upon challenge inoculation.

Methodology-

1. **Isolation of bacteria**

   Bacteria will be isolated from different soil sample.

2. **Maintenance of bacterial culture**

   Recovered bacterial isolates will be maintained on Nutrient agar media (NA) and plates will be incubated in the incubator at 28 ± 2°C for 24 to 36 hours. For long time preservation glycerol stock will be prepared using autoclaved glycerol + Nutrient broth cultures (1:1 v/v) and will be preserved in cryovials at –20°C.

3. **Maintenance of fungal culture**

   Fungal pathogens will be maintained on potato dextrose agar plates (PDA) and plates will be incubated in the incubator at 28 ± 2°C for 24 to 36 hours. For long time preservation glycerol stock will be prepared using autoclaved glycerol + Potato dextrose broth cultures (1:1 v/v) and will be preserved in cryovials at –20°C.

4. **Screening through functional characterization of PGP properties of bacterial strains**

   Screening of the different bacterial strains will be done by the functional characterization of bacteria for IAA production (Patten and Glick, 2002), ACC deaminase activity (Glick et al. 1999), phosphate solubilization (Diby et al. 2005) and siderophore production (Panhwar et al. 2012). Modification in the method will be done if required.

5. **Characterization of antifungal activity of bacteria**

   Antifungal activity will be detected using dual plate assay on PDA+NA plate and volatile organic compounds (VOCs) production assay.

6. **Seed pathogenicity assay of bacterial and fungal culture**

   Effect of bacterial and fungal culture will be tested on the soybean seed germination with different formulation.

7. ***In vitro* analysis of Plant Growth Promoting activities of bacteria on soybean plant**

   Effect of bacterial culture on the growth of soybean plant will be tested by two methods, seed bacterization and culture inoculation with different formulation in plant growth chamber and different growth parameter such as root, shoot length, number of lateral roots, fresh and dry weight of plant will be checked.

8. **Induction of defense-related genes in soybean plant**

Soybean seeds will be bacterized with different bacterial culture or plants will inoculated with bacterial culture broth with different formulation and then challenge inoculated with fungal pathogens for the induction of different defense related gene. Plants will be kept in plant growth chamber for different time period incubation with 16h/8h light/dark photoperiod.

9. **Characterization of defense related proteins in PGPB treated plants**

Crude protein extract will be prepared from different parts of plant according to Abdel-Monaim (2012) method and enzyme activity detection of different defense related protein such as PAL (Mori et al. 2001), LOX, POD (Choudhary 2011), PPO (Cavalcanti et al 2007) β-1, 3-glucanase (Pan et al. 1989) will be done in different part of soybean plants treated with bacterial culture with or without challenge inoculation and will be compared with control treatment. Isoform analysis will be done for defense enzymes.

10. **Molecular characterization of plant growth promoting (PGP) activities of bacteria**

Genomic DNA will be extracted from the bacteria and amplified with specific primer for different PGP activities such as nitrogen fixation, gluconic acid, ACC deaminase, chitinase using PCR thermal cycler. PCR product will be run on agarose gel electrophoresis end band pattern will be observed for specific gene.

6. **Molecular gene expression analysis of defense-related genes in soybean plant**

Molecular gene expression analysis of defense-related genes in soybean plant will be done for defense related gene expression such as chi, pal2, lox, pod, ppo, def, vsp, pal in plants treated with different formulation of bacterial and fungal pathogen through Semiquantitative RT-PCR and qPCR.

11. **Identification of bacterial culture and submission of sequence**

The colony PCR will be performed to amplify 16s rRNA of bacterial strains using eubacterial universal primers (Weisburg et al. 1991) and the presence and yield of specific PCR product (16S rRNA gene) will be monitored on agarose gel electrophoresis and visualization by ethidium bromide staining and viewing on a UV Gel doc system. The amplified PCR product will be sequenced and BLAST will be run. The nucleotide sequence obtained will be submitted to the NCBI Gene Bank and accession number will be obtained.

12. Submission of bacterial culture for open access

Identified culture with plant growth promoting activities and antifungal properties will be submitted to IMTECH, Chandigarh/MCC, Pune for open access.

13. Publication of research results and thesis writing

Publication of research results in National and International journals, books, symposiums and conferences.

Findings-

1. The microbe selected in the study *Bacillus* sp. SJ-5 (MCC no. 2069) possess most of the plant growth properties (IAA production, Pi solubilization, ACC deaminase production, proteases production, amylase production, siderophore production, nitrogen fixation, exopolysaccharide production) and biocontrol properties (siderophore production, Chitinase, β-1, 3-glucanae production, protease production, antifungal VOCs).
2. *Bacillus* sp. SJ-5 possesses strong antifungal activity against *R.solani* and *F. oxysporum* to protect soybean plants.
3. SJ-5 found to be significantly promotes soybean plant growth in normal and stress condition.
4. Molecular characterization of PGP properties of SJ-5 confirmed the presence of all the PGP and biocontrol genes.
5. GC-MS analysis of the *Bacillus* sp. SJ-5 VOCs revealed that it releases some of the potent VOCs which play important role in plant growth promotion and protection from fungal pathogens.
6. Inoculation of *Bacillus* sp. SJ-5 in soybean plants along with challenge inoculation of fungal pathogen shown accumulation of JA which is required for elicitation of ISR. In addition, it also enhances production of defense-related proteins that are produces upon elicitation of ISR. Comparative study in all part of the plants shown highest expression in the root part i.e. primary site of pathogen infection.
7. Gene expression study of all the plants shows significant changes in genes responsible for plant defense against fungal pathogen upon prior inoculation with *Bacillus* sp. SJ-5.

Overall, present study on bacterial induced systemic resistance in Soybean against fungal pathogen concludes that *Bacillus* sp. SJ-5 is a potent PGPB and biocontrol agent that can protect Soybean plants against fungal pathogen as well as increase plant growth also. In contest of agricultural world, it can be apply as biofertilizer to increase soybean yields in terms of heath and quantity.

Significance of work-

- Main significance of this research work will be reduction in the plant diseases caused by fungal phytopathogens through suppression mechanisms by bacterial isolates.
- Bacterial isolates will be allowed to promote the growth of soybean plants in presence of fungal pathogens.
- Bacterial isolates will be used as biocontrol for sustainability of agricultural crops under biotic stress.

References-

1. Abdel-Monaim MF, Mamdoh Ewis Ismail ME, Morsy KM (2012) Induction of systemic resistance in soybean plants against *Fusarium* wilts disease by seed treatment with benzothiadiazole and humic acid. African J Biotechnol 11(10):2454-2465
2. Agrios GN (1988) Plant Pathology, 3rd Ed. Academic Press Inc., San Diego USA
3. Berger S, Bell E, Sadka A, Mullet JE (1995). *Arabidopsis thaliana AtVsp* is homologous to soybean *VspA* and *VspB*, genes encoding vegetative storage protein acid phosphatases, and is regulated similarly by methyl jasmonate, wounding, sugars, light and phosphate. Plant Mol. Biol. 27: 933–942.
4. Bonas U, Lahaye T, (2002) Plant disease resistance triggered by pathogen-derived molecules: refined models of specific recognition. Curr Opin Microbiol 5:44–50
5. Cartieaux FP, Nussaume L, Robaglia C (2003) Tales from the underground: molecular plant-rhizobacteria interactions. Plant Cell and Environment 26: 189-199.
6. Cavalcanti FR, Resendea MLV, Carvalhoc CPS, Silveirab, Oliveira JTA (2007) An aqueous suspension of *Crinipellis perniciosa* mycelium activates tomato defence responses against *Xanthomonas vesicatoria.* Crop Prot 26:729–738
7. Choudhary DK (2011)Plant growth-promotion (PGP) activities and molecular characterization of rhizobacterial strains isolated from soybean (*Glycine max* L. Merril) plants against charcoal rot pathogen *Macrophomina phaseolina.* Biotechnol Lett 33:2287–2295
8. Choudhary DK (2012), Microbial rescue to plant under habitat-imposed abiotic and biotic stresses. Appl Microbiol Biotechnol 96:1137–1155
9. Choudhary DK, Johri BN (2009) Interactions of *Bacillus* spp. and plants—with special reference to induced systemic resistance (ISR). Microbiol Res 164:493–513
10. Choudhary DK, Prakash A and Johri BN (2007) Induced systemic resistance (ISR) in plants: mechanism of action. Indian J. Microbiol 47:289–297
11. Common Names of Diseases, The American Phytopathological Society. (http://en.wikipedia.org/wiki/List_of_soybean_diseases)
12. Compant S, Duffy B, Nowak J, Cle´ment C, Ait Barka E (2005) Use of Plant Growth-Promoting Bacteria for Biocontrol of Plant Diseases: Principles, Mechanisms of Action, and Future Prospects. Applied and environmental microbiology, 71: 4951–4959
13. Diby P, Sarma YR, Srinivasan V, Anandaraj M (2005) *Pseudomonas fluorescens* mediated vigour in black pepper (*Piper nigrum* L.) under green house cultivation. Anal Microbiol 55 (3):171-174

14. El-Abady MI, Seadh SE, Attia AN, El-Saidy Aml EA (2008) Impact of foliar fertilization and its time of application on yield and seed quality of soybean. The 2th field crops conference, FCRI, AV, Giza, Egypt
15. Epple P, Apel K, Bohlmann H (1995). An *Arabidopsis thaliana* thionin gene is inducible via a signal transduction pathway different from that for pathogenesis-related proteins. Plant Physiol. 109:813–820.
16. Eulgem T, Somssich IE (2007). Networks of WRKY transcription factors in defense signaling. Current Opinion in Plant Biology 10:366–371.
17. Farmer EE, Johnson RR, Ryan CA (1992). Regulation of expression of proteinase inhibitor genes by methyl jasmonate and jasmonic acid. Plant Physiol. 98:995–1002.
18. Farmer EE, Ryan CA (1992). Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell 4: 129–134.
19. Gilbert GS, Parke JL, Clayton MK, Handelsman J (1993) Effects of an introduced bacterium on bacterial communities on roots. Ecol 74:840-854
20. Glick BR, Patten CL, Holguin G, Penrose DM (1999) Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial Col press, London
21. Hashem EA, Abdalla HE, Hussein YA, Abd-Elnabi MA (2009) In vitro selection of soybean callus resistant to *Fusarium oxysporum* metabolites. Res J Agric Biol Sci 5(4):588–596
22. http://www.pnbkrishi.com/soybean.htm
23. Kloepper JW, Leong J, Teintze M, Schroth MN (1980). Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. Nature 286: 885–886.
24. Kloepper JW, Lifshitz R, Zablotowicz RM(1989) Free living bacterial inocula for enhancing crop productivity. Trends in Biotechnology 7: 39-44.
25. Mauch-Mani B, Slusarenko AJ (1996). Production of salicylic acid precursors is a major function of phenylalanine ammonialyase in the resistance of *Arabidopsis* to *Peronospora parasitica*. Plant Cell 8: 203–212.
26. Mori T, Sakurai M, Sakuta M (2001) Effects of conditioned medium on activities of PAL, CHS, DAHP synthase (DS-Co and DS-Mn) and anthocyanin production in suspension cultures of *Fragaria ananassa.* Plant Science 160:355–360
27. Nehl, D.B., Allen, S.J., and Brown, J.F. 1996. Deleterious rhizosphere bacteria: an integrating perspective. Applied Soil Ecology 5: 1-20.
28. Nürnberger T, Brunner F (2002) Innate immunity in plants and animals: emerging parallels between the recognition of general elicitors and pathogen-associated molecules. Curr Opin Plant Biol 5:1–7

29. Osburn RM, Milner JL, Oplinger ES, Smith RS, Handelsman J (1995) E¡ect of *Bacillus cereus* UW85 on the yield of soybean at two field sites in Wisconsin. Plant Dis 79:551-556
30. Pan SQ, Ye XS, Kuć J (1989) Direct detection of beta-1,3-glucanase isozymes on polyacrylamide electrophoresis and isoelectrofocusing gels. Anal Biochem,182(1):136–140
31. Panhwar QA, Othman R, Rahman ZA, Meon S, Ismail MR (2012) Isolation and characterization of phosphate-solubilizing bacteria from aerobic rice African J Biotechnol 11(11):2711-2719
32. Park S-W, Kaimoyo E, Kumar D, Mosher S, Klessig DF (2007). Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. Science 318, 113–116.
33. Patten C, Glick B (2002) Role of *Pseudomonas putida* Indoleacetic Acid in Development of the Host Plant Root System. App and Environ Microbiol 3795-3801
34. Penninckx IAMA, Thomma BPHJ, Buchala A, Métraux J-P, Broekaert WF (1998). Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in *Arabidopsis*. Plant Cell 10:2103–2113.
35. Pieterse CMJ, Ton J & van Loon LC (2001) Cross-talk between plant defense signaling pathways: boost or burden? Agri Biotech Net 3:1–18
36. Pieterse CMJ, van Wees SCM, van Pelt JA, Knoester M, Laan R, Gerrits H,Weisbeek PJ, van Loon LC (1998). A novel signalling pathway controlling induced systemic resistance in *Arabidopsis*. Plant Cell 10: 1571–1580
37. Potter S, Uknes S, Lawton K, Winter AM, Chandler D, DiMaio J, Novitzky R, Ward E, Ryals J (1993) Regulation of a hevein-like gene in *Arabidopsis*. Mol. Plant-Microbe Interact. 6: 680–685.
38. Ramamoorthy V, Raguchander T, Samiyappan R (2002) Induction of defense-related proteins in tomato roots treated with *Pseudomonas fluorescens* Pf1 and *Fusarium oxysporum* f. sp. *lycopersici.* Plant Soil 239:55–68
39. Samac DA, Hironaka CM, Yallaly PE, Shah DM (1990) Isolation and characterization of the genes encoding basic and acidic chitinase in *Arabidopsis thaliana.* Plant Physiol. 93: 907–914.
40. Saskia CM vanWees, Luijendijk M, Smoorenburg I, Leendert C. van Loon and Pieterse CMJ(1999), Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* is not associated with a direct effect on expression of known defense-related genes but stimulates the expression of the jasmonate-inducible gene *Atvsp upon challenge.* Plant Molecular Biology, 41: 537–549
41. Seo, Dong-Jun, Nguyen D-M-C, Song Y-S, Jung W-J (2012) Induction of defense response against *Rhizoctonia solani* in Cucumber plants by endophytic bacterium *Bacillus thuringiensis* GS1. J Microbiol Biotechnol 22(3):407–415

42. Sharma A, Johri BN (2003) Combat of iron-deprivation through a plant growth promoting fluorescent *Pseudomonas* strain GRP3 in mung bean. Microbiol Res 158:77–81
43. Thomma BPHJ, Eggermont K, Penninckx IAMA, Mauch- Mani B, Cammue BPA, Broekaert WF (1998) Separate jasmonate-dependent and salicylic acid-dependent defense response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. Proc. Natl. Acad. Sci. USA 95: 15107–15111.
44. USDA report, 2013
45. Vaan loon LC (1997). Induced resistance in plants and the role of pathogenesis-related proteins. European Journal of Plant Pathology 103: 753–765
46. Vleesschauwer D, Ho¨Fte M (2009) Rhizobacteria-Induced Systemic Resistance. Advances in Botanical Research 51:223-281
47. Wasternack C, Parthier B (1997). Jasmonate-signaled plant gene expression. Trends Plant Sci. 2:302–307.
48. Wei L, Kloepper JW, Tuzun S (1991). Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. Phytopathology 81:1508–1512.
49. Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173:697–703
50. World Soybean Production 2010 (http://en.wikipedia.org/wiki/Soybean)
51. Yan Z, Reddy MS, Ryu CM, Mc Inroy JA Wilson M, Kloepper JW (2002) Induced systemic protection against tomato late blight elicited by PGPR. Phytopathol 92: 1329 1333